ABSTRACT

Most physiological processes in mammals follow daily oscillations. These circadian rhythms are driven by central oscillator located in the suprachiasmatic nucleus (SCN) of hypothalamus. The SCN coordinates rhythmical activity of the subsidiary peripheral oscillators distributed in many different tissues. In gastrointestinal system, the peripheral clocks and metabolism are closely linked. The mechanism of circadian oscillations is based on transcriptional-translational feedback loops, which drive rhythmic expression of the clock genes. The entrainment with external conditions is essential for proper function of the circadian clock. While the SCN is driven mainly by the light-dark cycle, synchronization of the peripheral clocks depend on many factors, such as feeding and fasting. The length of the light part of the day, i.e. photoperiod, changes throughout the year rapidly and circadian system has to adapt to the changes all the time. However, a mechanism of adjustment to the change in the photoperiod has not been fully understood.

The aim of this work was to elucidate the effect of change in the photoperiod on the central SCN clock and on the peripheral clock in the liver. Firstly, we focused on dynamics of adjustment of these clocks to the change from a long photoperiod, with 18 hours of light, to a short photoperiod, with 6 hours of light. The next aim was to elucidate whether photoperiodic modulation of the hepatic clock is mediated by direct SCN signaling, indirectly by changes in feeding, or by both mechanisms.

The C57Bl/6 mice were maintained under a long photoperiod. Three, five and thirteen days after the change from the long to the short photoperiod, clock gene expression profiles in the SCN and in the liver were determined. *Per1*, *Per2* and *Rev-erbα* expression in the rostral, middle and caudal part of the SCN was assessed by *in situ* hybridization, the expression of *Per2* and *Rev-erbα* in liver was determined by real-time RT-PCR. In the next experiment, mice fed *ad libitum* were subjected to a change from the short to the long photoperiod or to a restricted feeding regime which simulated the change in the photoperiod.

The data demonstrate that the expression profiles, desynchronized in the rostral, middle and caudal parts of the SCN under the long photoperiod, attained synchrony after transition to the short photoperiod. Adjustment to the short photoperiod was achieved mostly by phase-advancing the clock gene expression decline. The expression rhythms of

Per2 and *Rev-erbα* in the liver adjusted to the change to the short photoperiod differently; whereas *Per2* expression adjusted by advancing the expression decline, *Rev-erbα* expression by advancing the expression rise. These data indicate different mechanisms of adjustment to the change of the photoperiod in the central SCN clock and in the peripheral clock in liver.

Five days after the change from a short to a long photoperiod, *Per2* and *Rev-erbα* expression profiles in the rostral, middle and caudal part of the SCN were desynchronized and thus fully adjusted to the long photoperiod. The daily profiles in the SCN were not affected by changes in feeding regime. The peripheral liver clock was entrained to the change from the short to the long photoperiod five days after the transition. In mice maintained under the short photoperiod with 6 hour nighttime feeding regime simulating the long photoperiod, the expression profiles were shifted to the same phase as under the long photoperiod, however, their waveforms were not modulated accordingly. In case of the change from the short to the long photoperiod with food provided twice a day simulating the short photoperiod, both phase and waveforms of the clock gene expression were affected. Therefore, these data demonstrate that photoperiodic modulation of food intake might shift the phase of clock gene expression profiles, but does not affect their waveforms.

These results indicate that the central SCN and the peripheral liver clock are affected by photoperiod in a different way. Apparently, direct signals from the photoperiod-modulated SCN as well as the feeding related signals are likely involved in photoperiodic entrainment of the peripheral clock in the liver.